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Silver birch ability to refill fully embolised xylem conduits under tension

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Abstract

Whether trees have the ability to refill embolised xylem conduits and possibly to do it under tension, i.e. in a physiologically active state, has been a topic of research and debate for many years. However, direct experimental evidence for embolism refilling remains scarce. Combining traditional tree hydraulic methods (e.g. hydraulic conductance measurements on excised stems), new approaches (cut end of a branch placed in contact with water held in an agar matrix), and newer technology (X-ray microtomography), we tested the potential of silver birch branches to refill embolised xylem conduits even under negative water potential. The hydraulic method suggests that refilling of embolised xylem can occur even under negative pressure. The successful ability of birch to refill xylem was confirmed by X-ray microtomography. Furthermore, our results suggest that refilling is unlikely to be driven osmotically given the small difference measured between sap and the osmolality of the source water (tap water, agar). Finally, our results have direct influence on the method of choice when studying the vulnerability of silver birch to cavitation.

Key words: sapwood, refilling, embolism, methodology, X-ray microtomography, hydraulic, *Betula pendula*

INTRODUCTION

A tree's ability to refill embolised xylem conduits is a central question in predicting how they will be able to cope with drought. However, the issue remains highly controversial and while several mechanisms have been proposed to explain potential xylem refilling (Nardini et al., 2011), experimental evidence remains scarce, possibly due to the lack of widespread affordable methods. X-ray microtomography has been used to study embolism and refilling in excised branches and small saplings (e.g., Cochard et al., 2015). However, this method is not easily available, it is costly and limited to small tissues/trees.

Here we use a simple method relying on traditional hydraulic measurements to test for silver birch's ability to refill while under small tension, i.e., comparable to what a tree might encounter at night. The ability of birch to refill winter-embolised xylem in boreal conditions by creating positive pressure in the xylem during the sap season, i.e. the period in the springtime between soil thawing and bud burst, has been known for a long time (e.g., Merwin and Lyon, 1909; Westhoff et al., 2008). Refilling during sap season has been proposed to rely on root pressure and the presence of osmotically active compounds released in the xylem conduits (e.g., Merwin and Lyon, 1909; Westhoff et al., 2008). However, it is not known if birch can also refill fully embolised xylem conduits outside the sap season under tension and if the refilling can happen without root pressure. We hypothesize that: 1) birch branches can refill outside the sap season and without root pressure since earlier work on birch refilling during the sap season showed that hydrostatic pressure aboveground was playing a role in refilling (Westhoff et al., 2008); and 2) that the refilling is osmotically driven, since it has been

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hypothesized to be the force allowing for refilling under tension (Holbrook and Zwieniecki, 1999; Westhoff et al., 2008).

MATERIALS AND METHODS

To test whether birch branches can refill we conducted two hydraulic experiments: 1) refilling of fully embolized branches under mild positive pressure, and 2) refilling of fully embolized branches under mild tension. To test whether osmotic mechanisms were involved, we used fresh branches and branches where living cells were killed with heating treatment, and measured osmolality of the xylem sap after refilling. To verify our findings of refilling, a new set of samples was imaged with x-ray microtomography during refilling in the absence of positive or negative pressure.

Plant material

Silver Birch (*Betula pendula* Roth.*)* branches were sampled on randomly selected trees (number of replicate per measurements is given below) on Viikki and Kumpula campuses of the University of Helsinki (Helsinki, Finland) during winter but outside of the sap season.

The sampled branches were one centimetre in diameter and one meter in length at minimum. A segment of each branch was recut according to experimental need (see below). Branch water potential (Ψ) was measured with a pressure chamber (PMS-1000, PMS instruments, Albany, OR, USA) until they could be considered fully embolised and non-conductive, i.e. when their water potential was lower than -3MPa. Silver birch P90 (water potential at which 90% of the hydraulic conductance is lost) is -2.75 MPa (Herbette and Cochard, 2010).

To control if the refilling is an active process or could be achieved simply by capillary movement within the conducting elements, we sampled additional branches from the same trees and killed the living cells in the xylem prior to measurements. Freshly sampled branches were wrapped in alimentary cling film to avoid desiccation that could trigger cracks in the wood and placed in a drying oven at 65°C for 24 h to kill living cells (Stiller and Sperry, 1999). This method was preferred to microwaving the sample, as earlier trials had shown that microwaving was creating cracks in the wood. Furthermore, the heating did not dry the samples because they were covered with a film to prevent dehydration. An earlier study (Lintunen et al., 2017) demonstrated that using the same protocol, the measured water potential was not decreased by the heating.

Refilling under mild positive pressure

Hydraulic conductance was measured gravimetrically according to Sperry et al. (1988) on both fully embolised fresh branches and killed branches. Briefly, the branches were vertically attached to a laboratory stand, a water reservoir was connected with tubing to the top of the branches, creating an overhead pressure of 0.08 Bar. Water flow through the branches was measured with a balance. To ensure that water had to flow through the xylem during the measurements, phloem was peeled off at the point of contact between the branch and the tubing and PTFE tape was used to prevent leaks and direct contact of water with the phloem. If conductance was restored during the measurement in a reasonable time period, i.e. within a few hours, we interpreted this as embolism refilling occurring. We chose a limit of 12 hours for refilling, because given tens or hundreds of hours, any branches, even the dead ones, would refill passively by capillary forces when placed in contact with water (see Yang and Tyree 1992). Therefore, we expected that no refilling would take place in dead branches over such a short period. However, refilling of living branches during that period would show that refilling is an active process. The maximum vessel length in silver birch has been found to be approximately 16 cm (Cochard et al., 2010), while the average vessel length is less than 5 mm (Piispanen et al., 2003). To avoid the artefacts in hydraulic conductance measurements caused by cut-open vessels, 3 samples of at least 30 cm were used.

Refilling under tension

To create water tension for refilling, a 10 g L^{-1} agar (VWR chemicals) solution was created and it's osmolality was measured at 0.04 osmo/kg with a freezing point osmometer (Osmomat 030 cryoscopic osmometer, Gonotec, Berlin, DE). Once the solution had cooled down enough to avoid damaging the living cells, but before the agar solution solidified, we inserted the bottom of a twig to the solution vertically. The twigs were bench-dried to water potentials lower than –3.5 MPa. The living bark was removed to a few mm above the insertion. Thus, only the xylem was in contact with the agar. The twig was held in place with a holder. The top of the twig was left in contact with the ambient environment. The agar solution created a matrix holding the water at a water potential of approximately -0.1 MPa (based on its osmolality and in agreement with the experimental results shown by Ghashghaie et al. (1991) for water potential of agar solution), a value close to what birches would experience at night in boreal regions (Sellin and Kupper, 2005). This value is sufficient to exclude a refilling by capillary forces. Based on the Laplace equation ($\Psi = 2\lambda/r$ where Ψ is the water potential of the agar solution, λ is the surface tension of water (~0.07 N/m) and r is vessel radius, which is assumed to be of the order of 10 µm (Lintunen et al., 2010)), the water potential at which capillary forces cannot contribute to refilling anymore is of the order of magnitude -0.01 MPa in birch. Thus, refilling in these twigs had to take place against the negative water potential of the matrix and gravity. After 12 hours, the twig water potential and hydraulic conductivity of the sample placed in agar were measured (see above).

Xylem sap osmolality

The first droplets of the xylem sap from the twig were collected from the "hydraulic conductance" and "refilling under tension" experiments so that osmolality could be measured with a freezing point osmometer (Osmomat 030 cryoscopic osmometer). The osmolality of the xylem sap could potentially give information on the refilling mechanism. A high osmolality would be expected if refilling occurred by the so called "osmotic refilling mechanism", where solutes would be secreted by the living cells to the refilling vessels thus drawing water for refilling osmotically (Nardini et al., 2011; Tyree et al., 1999).

X-ray microtomography

To verify refilling of conduits, a full x-ray microtomography scan was conducted on a living fully-embolised non-conducting sample (Ψ < -4 MPa) and a fully-embolised non-conducting killed sample similarly to those used in the two refilling experiments described above. To avoid losing resolution by increasing the field of view, the samples were selected to be smaller in diameter than for the conductivity measurements: 5 mm for the living sample and 4.5 mm for the dead one. The sample had to be shortened to just above 10 cm of length to have them straight to avoid movement of the sample outside the axis of rotation of the microtomography equipment. After the initial full scan, the samples were put in contact with approximately 1 ml of water at the base of the vertically held twigs (i.e., no positive pressure). Low resolution scans were performed every 20 min for three hours. Four hours later, a second full scan was performed. The X-ray microtomography scans were conducted according to Suuronen et al. (2013). Briefly, desktop microtomography equipment (Nanotom 180 NF, Phoenix|x-ray Systems and Services, Wunstorf, Germany; currently owned by GE Measurement and Control Solutions) was used. One full scan was taken over a full 360° rotation of the sample. Image reconstructions were performed with datos|x –reconstruction software, provided by Phoenix|x-ray Systems and Services. Xylem embolised and refilled areas were calculated with ImagePro Plus (Media Cybernetics, Inc., Bethesda, MD, USA) based on the colour difference.

RESULTS

Refilling under mild positive pressure

The hydraulic conductivity recovered in living samples but not in killed ones (Figure 1), indicating that embolism refilled in living samples, and that living cells were important in

facilitating refilling. On average, the first drop of water came through the living samples 87 min after they were placed in contact with water (Figure 1). The hydraulic conductance increased until 300 min after the branch was put in contact with water, then it remained more or less steady until the measurement was stopped at 700 min after contact with water. No water came through the killed samples for the 700 min that the experiment lasted. In all these experiments, the osmolality of the xylem sap extracted was 0.0391 ± 0.010 osmo/kg.

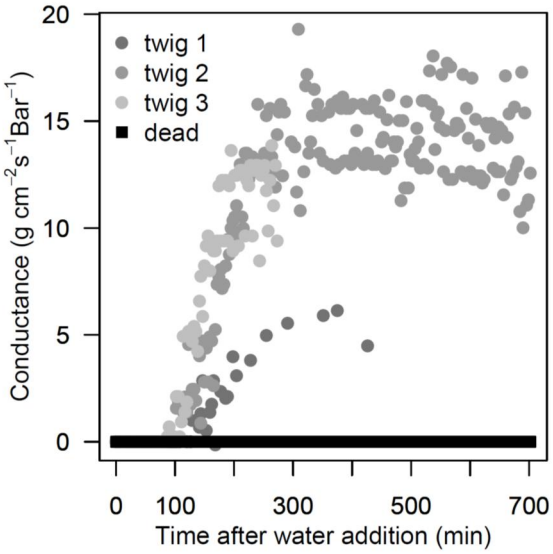

Figure 1. Hydraulic conductance of living (grey circles) and dead (black squares – however, it shows a line at y=0) birch branches after being placed in contact with water (n=3).

Refilling in agar

After being in contact with the agar overnight, water potential of three twigs were measured at 0.0 MPa (the top cut surface was wet), which is above the water potential (-0.1 MPa) of the agar matrix used as a source for water. When the refilled twig was put in contact with water in a traditional hydraulic conductance measurement setup (see above), it immediately started to conduct water (Figure 2). The osmolality of the xylem sap extracted was 0.055 ± 0.002 osmo/kg.

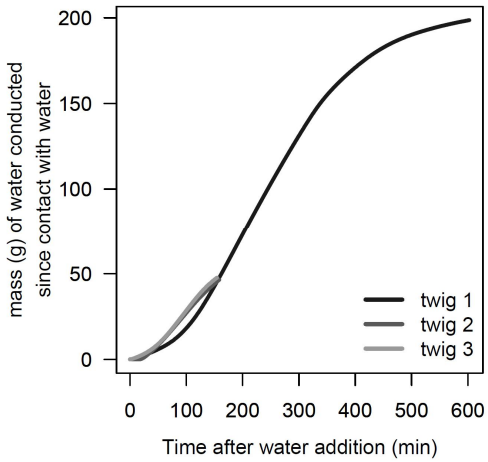

Figure 2: Mass of water flowing through living branches refilled in agar (at -0.1 MPa) through time. Time 0 indicates the time the branches are placed in contact with water.

X-ray microtomography

Compared to the fully embolised branch prior to water contact, the living sample showed a 97 % decrease in embolised xylem area four hours after the contact with water (Figure 3 A and B), while the killed sample showed no decrease in embolised xylem area (Figure 3 C and D), confirming our findings that embolism recovery occurred in the living sample, but not in the killed one.

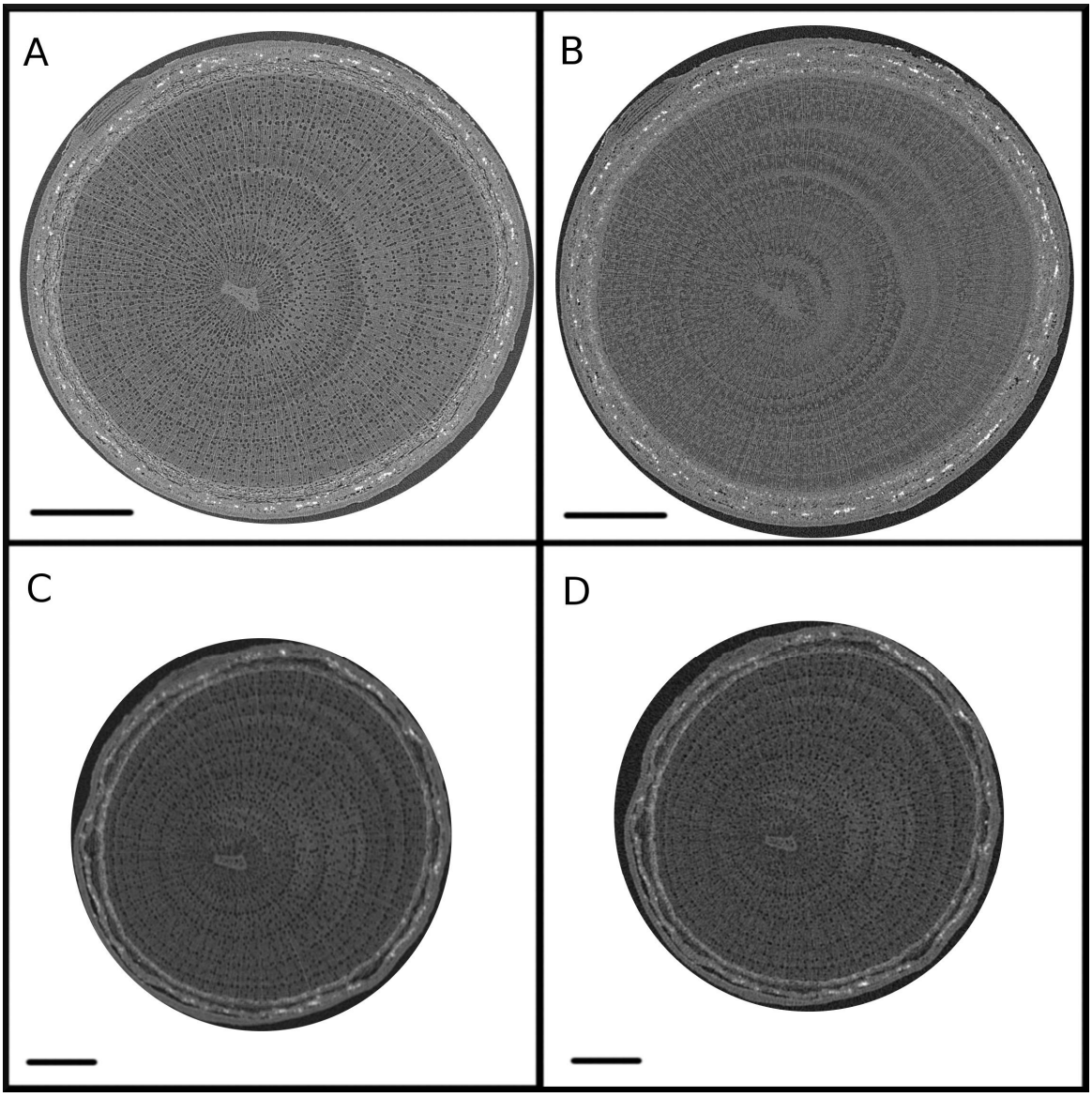

Figure 3. X-ray tomography cross-section of a living and dead birch twig tested for refilling. Living branch is shown fully embolised (A) and four hours after the xylem at the bottom of the twig has been placed in contact with water (B). Dead branch is shown fully embolised (C) and four hours after the xylem at the bottom of the twig has been placed in contact with water (D). Higher density materials appear whiter on the picture, with black dots in the sapwood showing embolised xyelm conduits, while grey ones (not always easy to distinguish from the rest of the tissue) show conduits filled with water. Black bar represents 1mm.

DISCUSSION

Our results show that excised branches of silver birch successfully refilled fully-embolised non-conductive xylem conduits and restored hydraulic conductivity. Furthermore,

a birch twig was able to refill from -4 MPa to 0 MPa under a small negative water potential (i.e., under tension) similar to what a tree might experience at night.

Working with excised branches to study refilling has recently been challenged (e.g., Knipfer et al., 2017; Wheeler et al., 2013). It has been suggested that the refilling in excised branches occurs due to capillary relocation of water, and that such refilling does not take place in intact trees. We argue that this issue is not likely to be a problem when working with birch branches, and that our results on cut branches can be extrapolated to the whole tree for the following reasons. Firstly, as stated above, the ability of birch to refill winter-embolised xylem by creating positive pressure in the xylem in spring has been known for a long time (e.g., Merwin and Lyon, 1909). It has been shown that in mature birches, both root pressure and pressurisation of the sap at the top of the trees are involved (Westhoff et al., 2008). Secondly, earlier work as shown that intact partially embolised – in contrast to our fully embolised branches - birch saplings have been shown to refill (Suuronen et al., 2013). Thirdly, we prove that the refilling as observed here cannot be the result of purely physical processes, such as capillarity for three reasons: 1) Refilling of fully embolised xylem by capillary forces would take tens of hours (Yang and Tyree, 1992), much longer than the time observed here even with the refilling under negative water potential, 2) The capillary rise cannot be responsible for the refilling at -0.1 MPa as it should theoretically be stopped at ~ -0.01 MPa, and 3) Refilling did not occur in killed samples.

Contrary to our hypothesis, xylem sap osmolality was found to be rather small, corresponding to a water potential of ~ -0.1 MPa. This hints towards refilling not occurring by the "osmotic refilling mechanism", since the water potential of the xylem sap collected was only slightly more negative than the water potential of the agar solution. However, it has been proposed that xylem sap osmolality could be involved in the refilling without a bulk increase in sap osmolality, because the solutes would be taken back by the living cells shortly after the refilling was completed (Wegner, 2014), but experimental support for such a process is lacking.

Our results also have practical implication for studying birch hydraulic properties, and in particular to construct xylem vulnerability curves for birch. Traditional methods, such as hydraulic conductance measured by putting the sample in contact with water under a slight positive pressure, led to the restoration of the hydraulic conductance within less than 90 minutes in our experiment even for samples that were fully embolised. Hence, building PLC (Percent loss conductivity) curves proved impossible for birch with traditional methods. More modern methods, such as the Cavitron (Cochard et al., 2005) or X-ray microtomography (Cochard et al., 2015) are likely better suited to study the loss of hydraulic conductance in birch branches. In the Cavitron method, the constant force applied under tension through spinning might prevent or delay the refilling on embolised vessel, while conductance calculation based on the amount and dimension of embolised vessels in a twig obtained with X-ray microtomography can be done without putting the sample into contact with water, therefore without triggering refilling.

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